Study of Bone Marrow Changes During the First Months of Treatment

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Seventeen patients with hairy cell leukemia (HCL) were treated with low doses of recombinant alpha interferon (IFN) for over 4 months. Marked improvement was observed in peripheral blood and bone marrow in 15 of 17 patients. Comparison of pretreatment values and hemograms obtained after 4 months of treatment showed a marked decrease in circulating hairy cells (P < .01), a decrease in the number of lymphocytes (P < .01), a rise in the number of platelets (P < .05), granulocytes (P < .05), and monocytes (P < .01), and a rise in the hemoglobin level (P < .01). Transient reduction in the number of granulocytes was noted during the first month. Correction of thrombocytopenia often appeared within 2 months and usually preceded improvement in monocytes, lymphocytes, and neutrophils. Bone marrow biopsy specimens were taken before treatment and 2, 4, and 7 months after its initiation. The volumes occupied by hairy cells, cells of the myeloid lines, and adipocytes were studied by stereological analysis of semithin sections. Decrease in the volume occupied by hairy cells was seen after 4 months of treatment (P < .01), and the volume continued to decrease at the seventh month (P < .05). Hairy cells were no longer detected on bone marrow biopsies of 4 of 17 patients by the fourth month and in 3 of 8 additional patients by the seventh month. A rise in the volume occupied by normal myeloid cells was visible by the second month of treatment (P < .01). Nevertheless, the volume occupied by granulocytes remained lower than in the normal controls (P < .01).

After an initial increase during the first 2 months of treatment (P < .01), the overall cellularity remained unchanged at 4 months and decreased significantly (P < .05) at 7 months. Except for biopsies at 2 months, mean cellularity was below that of control biopsies (P < .01).

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RESULTS

After 4 months of treatment, we found a decrease in the number of circulating hairy cells \((P < .01)\) and lymphocytes \((P < .01)\), platelets \((P < .05)\), and hemoglobin \((P < .01)\). Normalization of anemia (Hb < 12.5 g/dL), granulocytopenia (granulocytes < 1.5 \times 10^9/L), and thrombocytopenia (platelets 150 \times 10^9/L) was observed, respectively, in 8 of 11, 6 of 12, and 7 of 10 of the initially affected patients. Figure 2 shows the evolution of the mean hematologic values over the first 4 months. During the first month there was a decrease in the number of circulating hairy cells, particularly marked (with the exception of one nonresponder patient) for the hyperleukocytic patients. There was also a transient reduction in granulocytes for all patients during the first month. As shown in Fig 2, in initially cytopenic patients, mean platelet levels increased the first and second months of treatment, while increases in granulocytes and hemoglobin levels were not significant before the end of the second month. The evolution of monocyte levels was somewhat variable, with transient monocytosis commonly cumulating during the third month. In patients who were not initially cytopenic, no significant changes in neutrophil, platelet, or hemoglobin levels occurred after the second month (Fig 2).

In the 13 patients who were treated for over 4 months, and results remained good during continued treatment (5 to 7 months) or even improved (five cases). Hairy cells were no longer detectable in 4 of 17 patients (all treated with alpha2 IFN) after 4 months and in 3 of 8 additional patients (1 treated with alphaA IFN) after 7 months. All biopsies showed reticular fibrosis. After 4 months of treatment, we observed no clear fibrosis diminution as assessed by conventional morphology. Fibrosis was

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**Fig 1.** Semithin section of bone marrow biopsy obtained after 4 months of treatment. —— hairy cells; —— erythroblasts; —— granulocytes.

Statistically, 1,000 points were counted \((1,010 \pm 44)\), giving a probable error of .2%, .3%, .5%, and .7% for the relative volumes (ie, volume density) of 1%, 2%, 5%, and 10%, respectively. The analysis was done on randomly selected fields according to a predetermined diagram. With counting based on the entire cell (both nucleus and cytoplasm), each cell was classified as one of the following: hairy cell, erythroblast, megakaryocyte, granulocyte, or adipocyte. Lymphocytes, plasmocytes, eosinophils, vascular cells, and unidentifiable cells were grouped together in the category “other cells.” Extracellular spaces and other elements of the stroma were also computed. As extracellular spaces and fibrosis were obvious in most biopsies, cellularity cannot be defined by reference to adipocytes. We defined cellularity as the sum of hairy cell, myeloid cell, and “other cell” relative volumes. Ten normal-appearing marrows were measured as controls (Hodgkins and non-Hodgkins lymphomas without bone marrow infiltration in adults aged 40 to 50 years).

**Statistical analysis.** Comparisons of the means were tested by Wilcoxon's paired test for paired values and by Mann-Withney's test for unpaired data. Correlations were analyzed by the Spearman correlation test. The \(P\) level was set at 5%. Data were pooled from patients treated with alphaA IFN and from those treated with alpha2 IFN. For simplicity, both interferons are referred to as IFN in the text.

**RESULTS**

After 4 months of treatment, we found a decrease in the number of circulating hairy cells \((P < .01)\) and lymphocytes \((P < .01)\) and a rise in the level of granulocytes \((P < .05)\), monocytes \((P < .01)\), platelets \((P < .05)\), and hemoglobin \((P < .01)\). Normalization of anemia (Hb < 12.5 g/dL), granulocytopenia (granulocytes < 1.5 \times 10^9/L), and thrombocytopenia (platelets 150 \times 10^9/L) was observed, respectively, in 8 of 11, 6 of 12, and 7 of 10 of the initially affected patients. Figure 2 shows the evolution of the mean hematologic values over the first 4 months. During the first month there was a decrease in the number of circulating hairy cells, particularly marked (with the exception of one nonresponder patient) for the hyperleukocytic patients. There was also a transient reduction in granulocytes for all patients during the first month. As shown in Fig 2, in initially cytopenic patients, mean platelet levels increased the first and second months of treatment, while increases in granulocytes and hemoglobin levels were not significant before the end of the second month. The evolution of monocyte levels was somewhat variable, with transient monocytosis commonly cumulating during the third month. In patients who were not initially cytopenic, no significant changes in neutrophil, platelet, or hemoglobin levels occurred after the second month (Fig 2).

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always diffuse. In the biopsies showing hairy cell focal invasion, fibrosis seemed greatest in the regions infiltrated by these neoplastic cells. Compared to the initial biopsies, after 4 months all patients showed a significant decrease in the volume occupied by hairy cells (P < .01) and an increase in the volumes occupied by cells of the granulocyte (P < .01), erythroblast (P < .01), and megakaryocyte (P < .01) lines. No significant changes of volume were found for adipocytes, but a significant increase in overall cellularity (P < .05) was observed.

The evolution of the mean stereological parameters is illustrated in Fig 3. A significant decrease (P < .01) in the volume occupied by hairy cells was found in month 4 biopsies and became more marked by month 7 (P < .05). An increase in the volumes occupied by myeloid cells became distinct by the second month (P < .01), but month 4 biopsies showed no further significant change. Month 7 biopsies showed a decrease in the volumes of erythroblasts (P < .05), while granulocytic and megakaryocytic cell lines showed no significant changes. The mean volumes occupied by adipocytes remained comparable on initial biopsies and after 2 and 4 months' treatment. By the seventh month, however, an increase was observed in adipocyte volume compared to the month 4 volume for the same patients (P < .01). Mean granulocyte volumes, either initially or after 2, 4, and 7 months of treatment (respectively, 2.94 ± 4.79%, 11.12 ± 8.47%, 13.66 ± 8.10%, and 12.86 ± 8.88%) remained significantly (P < .01, U test) below those of controls (26.11 ± 3.68%). This hypoplasia of the granulocytic line was not correlated with the persistence or disappearance of hairy cells and was present in 4 of 7 patients whose biopsies no longer showed any hairy cells. The cellularity increased after 2 months (P < .01), remained unchanged after 4 months, and decreased after 7 months (P < .05). No statistically significant correlation (Spearman test) was found between platelet, neutrophil blood cell count, hemoglobin level, and BM volumes of megakaryocytic, granulocytic, and erythroblastic series, respectively.

As a whole, a clear improvement was observed for all but two patients. No improvement was noted for one patient, who was characterized by the presence of hyperleukocytosis, the absence of any myeloid cytopenia with a persistent monocytosis appearing after splenectomy. The response was modest for another patient who had undergone unsuccessful chemotherapy (vinblastine, bleomycin, prednisolone), which was discontinued 1 month prior to treatment with alpha IFN. With the 15 other patients, regardless of the form of alpha IFN administered, distinct improvement of blood, bone marrow, or both was obtained. The splenomegaly present in four patients disappeared during the first 2 months of treatment. It must be stressed, however, that complete normalization of blood and bone marrow parameters (including restoration of myeloid volumes at normal level) were obtained in only one patient. Apparently, splenectomized and nonsplenectomized patients show identical outcomes during the first months of therapy.

**DISCUSSION**

The management of HCL is still open to controversy. Splenectomy produces improvement of myeloid cytopenias in 30% to 60% of cases and significantly increases survival for patients with severe splenomegaly.6,7 What remains difficult is the treatment of patients without splenomegaly (28% of 211 cases in our previous series) and of nonresponders to splenectomy (50% in our series). Single-drug therapies, particularly with chlorambucil, often improve anemia and thrombocytopenia but seem to have no effect on neutropenia;6 the patients remain exposed to infection, which is the most common cause of death and an important prognostic factor.7 Leukapheresis6 produces only transient improvement. Combination drug therapy is used for the more serious cases (approximately 5% of all cases) and can induce some complete remissions, but at the cost of very high mortality.10 Quesada’s recent demonstration of the efficacy of natural alpha IFN11 prompted us to start a clinical trial with recombinant alpha IFN for patients with HCL of varying degrees of severity. The unequivocal effects documented in the blood and bone marrow of 15 of 17 patients who were monitored for more than 4 months attest to the rapid action of alpha IFN treatment and are in agreement with results of other concurrent studies.11-16 Compared to other forms of treatment (other than combination drug therapy), IFN is the only one that achieves the disappearance of hairy cells in the bone marrow in a certain percentage of cases (4 of 17 cases after 4 months and 3 of 8 additional cases after 7 months) and the correction of myeloid cytopenia.

The difficulty of correctly assessing the state of bone marrow during treatment must be stressed. Reticular fibrosis, which interferes with aspiration, persists for several months even after complete remission.6 Hairy cell infiltration can be studied only by using bone marrow biopsies, and even...
then, cell identification remains difficult and presents diagnostic problems when the infiltration is moderate. The technique of semithin sections prepared from specimens embedded in resin makes the identification of abnormal cells easier (Fig 1) and permits reproducible stereological studies of the volumes occupied by the different marrow cells. This objective quantitative analysis allows us to establish that the increase in the myeloid cell volumes is rapid, detectable by the end of the second month of treatment. The decline of the hairy cell infiltration generally occurs later, becomes significant only by month 4, and continues to decline until month 7. These results are in contrast with those of the peripheral blood, in which the decrease of hairy cells occurred early, often within the first month, whereas correction of cytopenia (except thrombocytopenia) takes place later, becoming significant only after 3 or 4 months of treatment. The dissociation of blood and marrow results can be observed both at month 4 and at month 7 and compels us to evaluate the treatment by its effects on blood and bone marrow alike.

Quantitative analysis of the biopsies also allows us to show that the volumes occupied by the granulocytic series remained much smaller than in the controls (except for two of 17 cases at month 4 and one additional case of eight at month 7). In addition, 7-month biopsies showed a decrease in cellularity and erythroblast volumes and a clear increase in adipocyte volume. The significance of these findings remains unclear. Both residual disease and interferon therapy may be involved. However, a biopsy represents but a very small fraction of the total marrow. Marrow scans will be performed to search for a peripheral expansion of active hematopoietic marrow. This fact can explain the lack of correlation between blood counts and volumes occupied by the myeloid precursor cells in the bone marrow.

The quality of the clinical results in the long term, the advantage of obtaining complete remission rather than mere normalization of peripheral cytopenias, and the optimum duration of treatment still need to be clarified. Nevertheless, it is already possible to assert that the results obtained to date for the more severe forms (eg, severe pancytopenia with serious recurrent infections: three cases in this study) are of considerable interest since the prognosis without treatment for such patients is poor. Furthermore, the very low growth rate of hairy cells17 gives us good reason to hope for lengthy remissions for a high proportion of HCL patients.

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Treatment of hairy cell leukemia with recombinant alpha interferon: I. Quantitative study of bone marrow changes during the first months of treatment

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